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Date: March 4, 2002 By: Lynn Anderson

DOCKET NO.: 0450-0311.31

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF:	
Iversen	EXAMINER: J. Epps
SERIAL NO.: 09/574,570	ART UNIT: 1635
FILED: May 17, 2000	
FOR: **Enzyme Inhibitors for Metabolic Redirection**	

DECLARATION UNDER 37 C.F.R. §1.132

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

I, Patrick L. Iversen, declare and affirm as follows:

I am currently employed as Senior Vice President of Research and Development at AVI Biopharma Inc. I have been employed at AVI Biopharma, previously known as Antivirals Inc., since 1997.

I received a Ph.D. in the field of Biochemical Pharmacology and Toxicology from the University of Utah in 1984.

I supervised, participated in, and/or have firsthand knowledge of the studies reported below.

Enhanced Cytotoxicity of Paclitaxel Co-administered with PMO Antisense to CYP3A4

Cell viability of primary human hepatocytes and caco-2/h3A4 cells (human colon carcinoma cell line caco-2 transfected with CYP3A4 cDNA on an extrachromosomal vector p220CMV3A4; Gentest, Woburn, MA) was assessed following co-treatment with a phosphorodiamidate-linked

morpholino oligomer (PMO) having the sequence presented in the above-referenced application as SEQ ID NO: 47 (targeted to a sequence of human CYP3A4 mRNA containing an AUG start codon), or a control PMO, in combination with three cytotoxic drugs: paclitaxel, cyclophosphamide and cisplatin. Of these, the first (paclitaxel) is metabolized to less cytotoxic metabolites by CYP3A4, the second (cyclophosphamide) is a prodrug that requires metabolic activation by CYP3A4 to become cytotoxic, and the third (cisplatin) is not metabolized by CYP3A4.

Cells were treated with PMO 24 hours prior to addition of the cytotoxic drugs (5 μM paclitaxel, 600 μM cyclophosphamide, or 7 μM cisplatin). Cell viability was determined by MTT assay after an additional 24 hours incubation.

Results:

Addition of the antisense PMO having sequence SEQ ID NO: 47 in combination with paclitaxel reduced cell viability in both model systems, as would be expected from greater exposure of the cells to unmetabolized paclitaxel.

Co-treatment of cells with the antisense PMO and cyclophosphamide significantly increased the cell viability in both model systems compared to treatment with cyclophosphamide alone, as would be expected from decreased metabolic activation of the prodrug.

Co-treatment of cells with cisplatin and the antisense PMO did not significantly alter cell viability from this moderately cytotoxic toxic dose of cisplatin.

Cell Cycle Study of PMO/Paclitaxel Treated Cells:

Additional studies were carried out to investigate the mechanism of alteration of paclitaxel toxicity by antisense PMO in caco-2/h3A4 cells. Cells were treated with control PMO or antisense PMO (SEQ ID NO: 47, as above) 24 hours prior to addition of 5 μM paclitaxel. After an additional 24 hours paclitaxel incubation, the cell cycle distribution of the caco-2/h3A4 cells was determined by flow cytometry.

The addition of paclitaxel and control PMO to the cells increased the percent of viable cells in G_1/G_0 phase, a cell cycle checkpoint indicative of DNA damage. It is likely that the DNA damage was produced predominantly by the 3'-(p-hydroxyphenyl)taxol metabolite produced by CYP3A4.

Conversely, viable caco-2/h3A4 cells treated with paclitaxel and antisense PMO (SEQ ID

NO: 47) accumulated largely in the S phase of the cell cycle, indicating a release of the G_1-S DNA damage checkpoint and a return to paclitaxel's mechanism of action at the microtubule level.

The above results provide further evidence that the method of the invention of claim 1, employing morpholino antisense oligomers targeted to p450-encoding RNA, is effective to increase effectiveness of a drug, in this case Taxol, by inhibiting its metabolism to less effective metabolites. These particular results are further pertinent to the subject matter of dependent claims 12-14 and 21-24.

I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Respectfully submitted,

Feb. 27, 2002
Date

Patrick L. Iversen, Ph.D.